Figure 1A

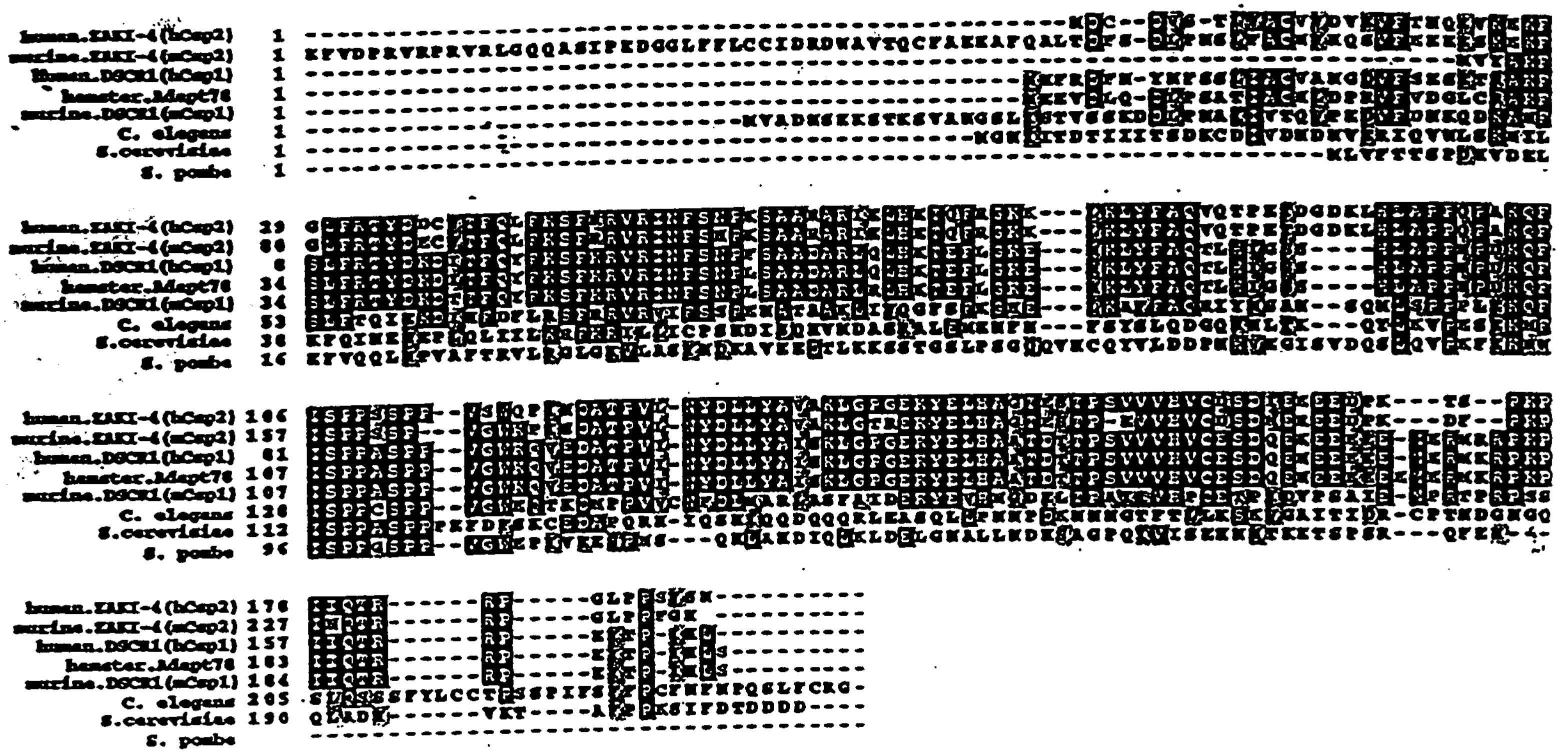
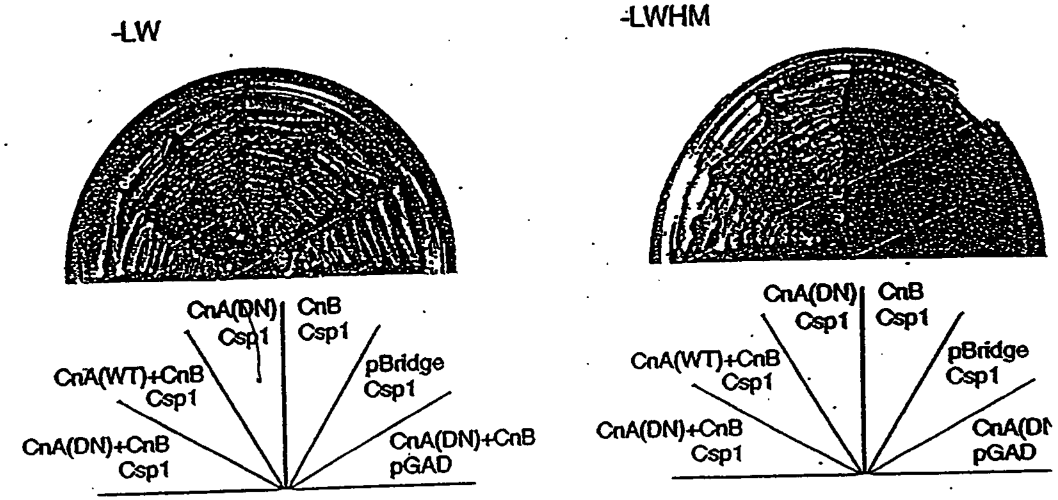

human.ZAKI-4(hCsp2) 1
murine.ZAKI-4(mCsp2) 1
human.DSCR1(hCsp1) 1
hamster.Adapt78 1
murine.DSCR1(mCsp1) 1
C. elegans 1
S.cerevisiae 1
S. pombe 1
human.ZAKI-4(hCsp2) 29
murine.ZAKI-4(mCsp2) 80
human.DSCR1(hCsp1) 8
hamster.Adapt78 34
murine.DSCR1(mCsp1) 34
C. elegans 53
S.cerevisiae 36
S. pombe 16
human.ZAKI-4(hCsp2) 106
murine.ZAKI-4(mCsp2) 157
human.DSCR1(hCsp1) 81
hamster.Adapt78 107
murine.DSCR1(mCsp1) 107
C. elegans 126
S.cerevisiae 112
S. pombe 96
human.ZAKI-4(hCsp2) 176
murine.ZAKI-4(mCsp2) 227
human.DSCR1(hCsp1) 157
hamster.Adapt78 183
murine.DSCR1(mCsp1) 184
C. elegans 205
S.cerevisiae 190
S. pombe

Figure 1B



-LW
CnA(DN)
Csp1
CnB
Csp1
CnA(WT)+CnB
Csp1
pBridge
Csp1
CnA(DN)+CnB
Csp1
CnA(DN)+CnB
pGAD
-LWHM
CnA(DN)
Csp1
CnB
Csp1
CnA(WT)+CnB
Csp1
pBridge
Csp1
CnA(DN)+CnB
Csp1
pGAD

Figure 2

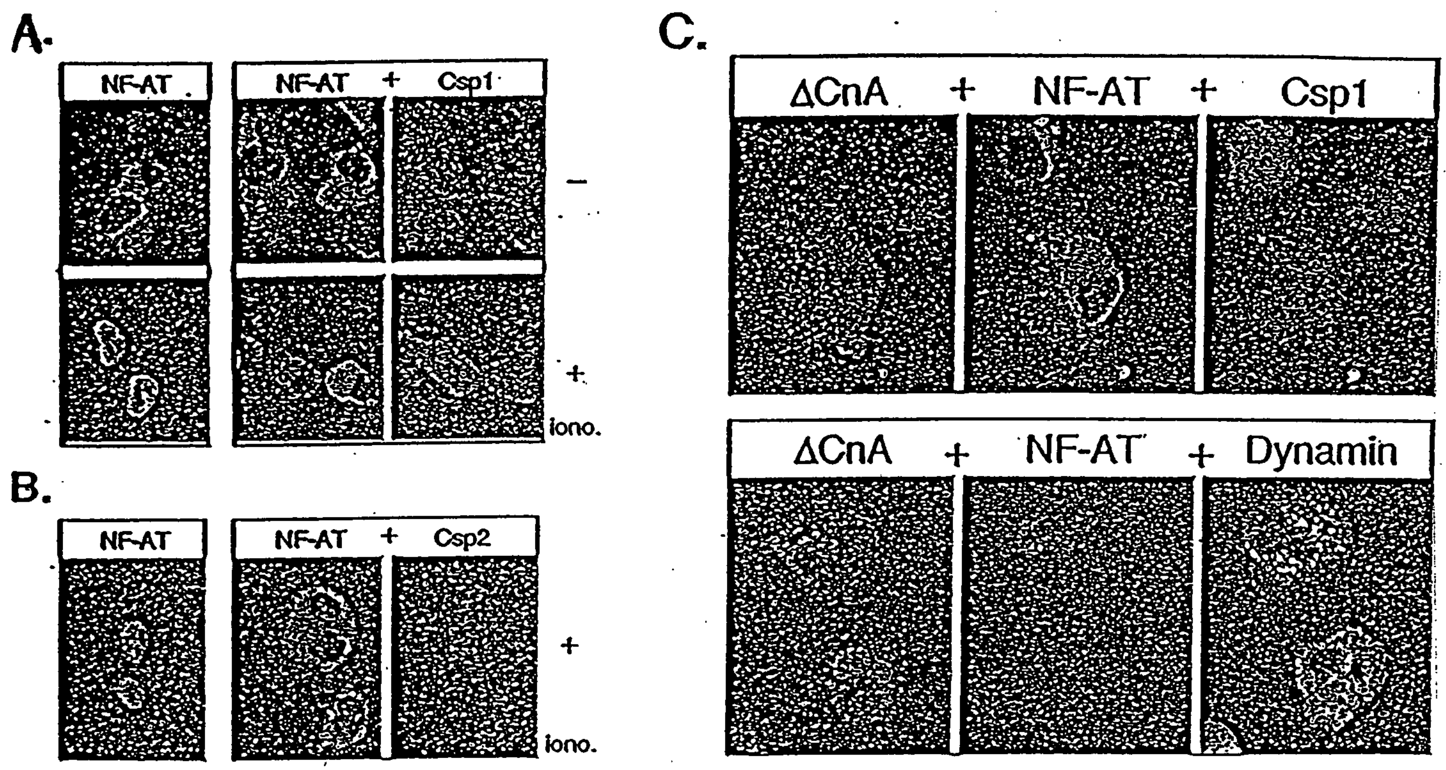
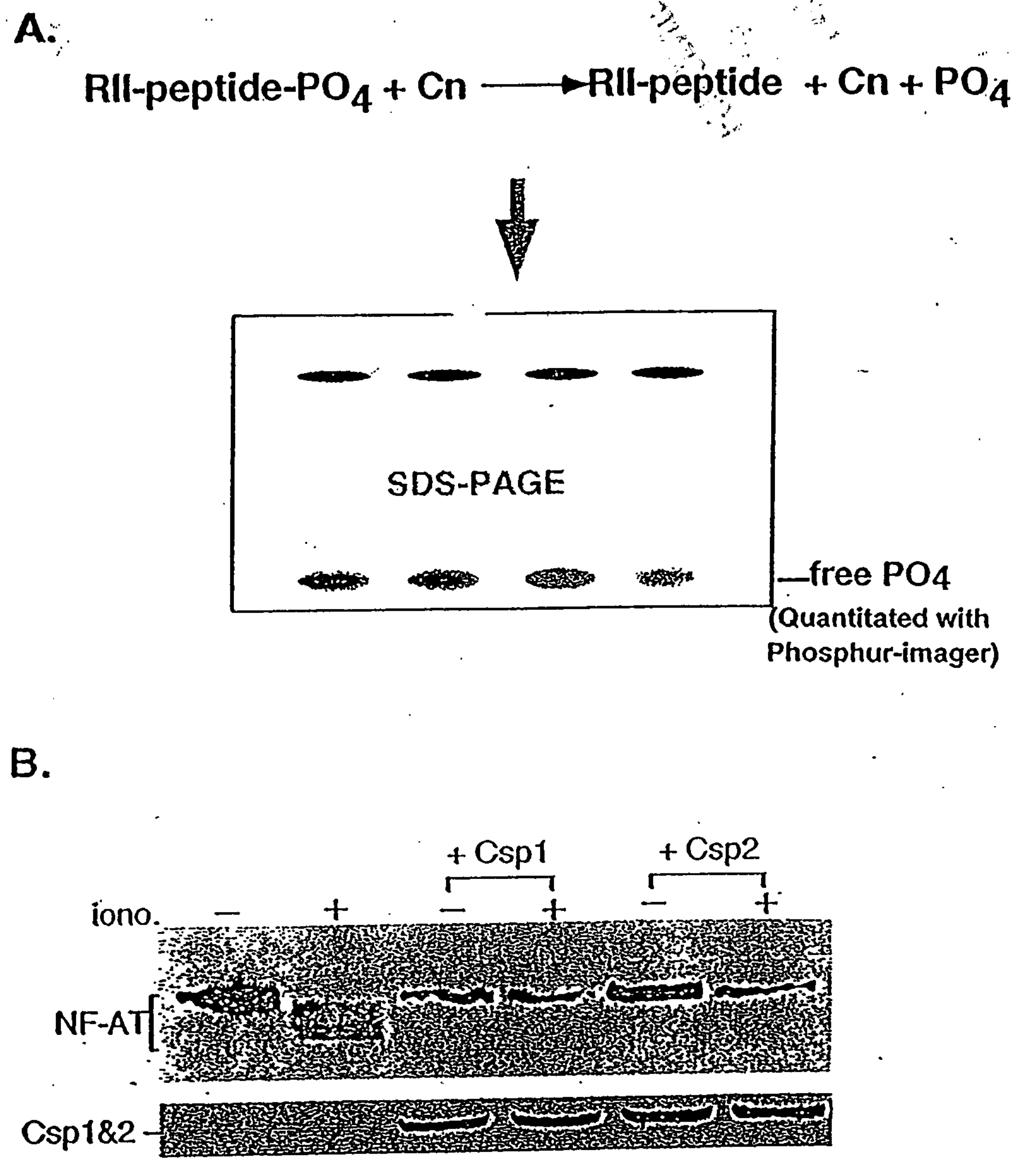
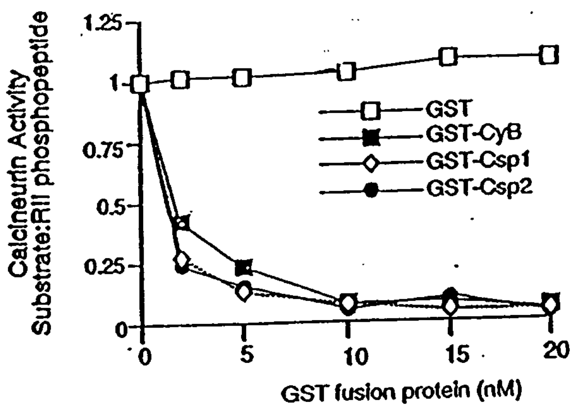

A.
NF-AT
NF-AT + Csp1
−
+
iono.
B.
NF-AT
NF-AT + Csp2
+
iono.
C.
ΔCnA + NF-AT + Csp1
ΔCnA + NF-AT + Dynamin

Figure 3


A.
RII-peptide-PO4 + Cn → RII-peptide + Cn + PO4
SDS-PAGE
—free PO4
(Quantitated with
Phosphur-imager)
B.
+ Csp1
+ Csp2
iono. – + – + – +
NF-AT
Csp1&2

A.

Figure 4


Calcineurin Activity
Substrate:RII phosphopeptide
1.25
1
0.75
0.5
0.25
0
0
5
10
15
20
GST fusion protein (nM)
GST
GST-CyB
GST-Csp1
GST-Csp2

B.

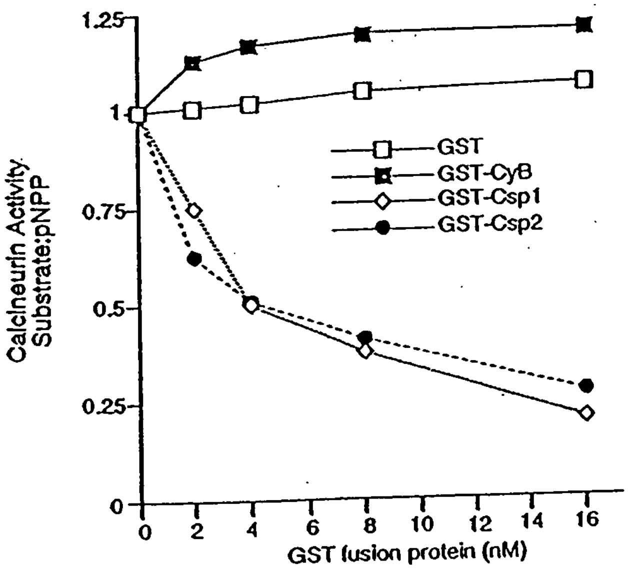

Calcineurin Activity
Substrate:pNPP
1.25
1
0.75
0.5
0.25
0
0
2
4
6
8
10
12
14
16
GST fusion protein (nM)
GST
GST-CyB
GST-Csp1
GST-Csp2

Figure 5

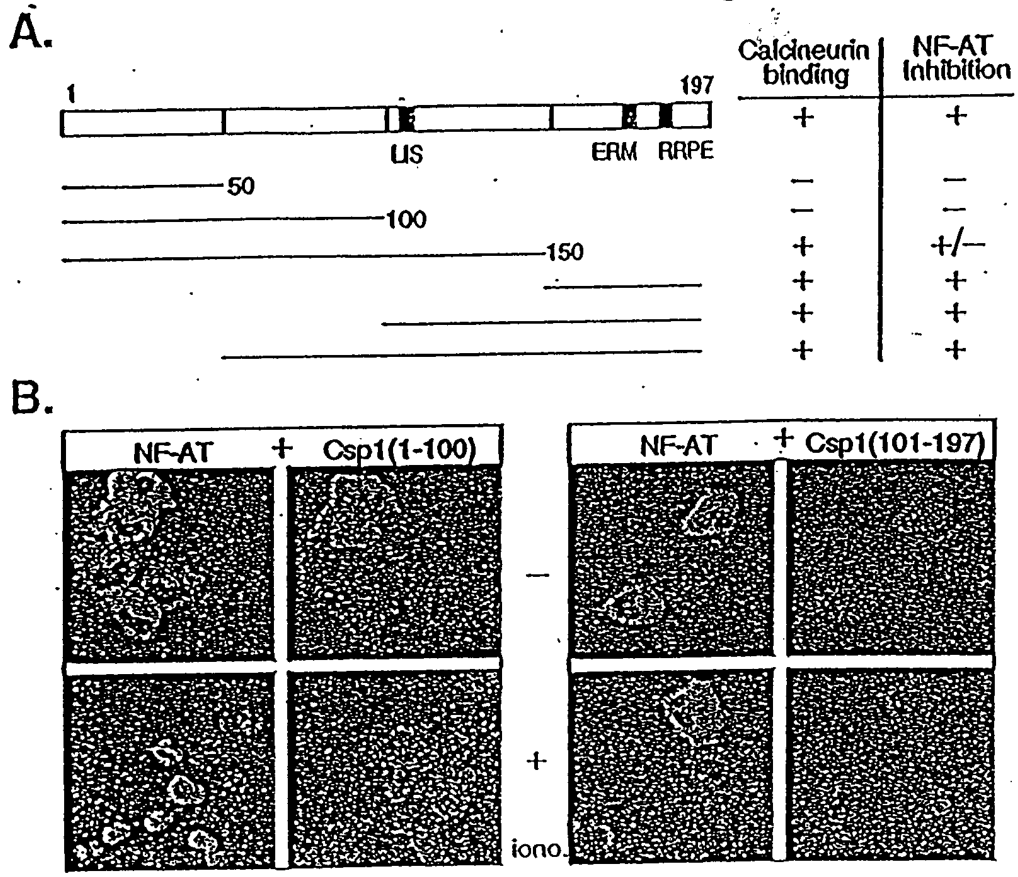

A.
1
197
LIS
ERM
RRPE
50
100
150
Calcineurin binding
NF-AT Inhibition
B.
NF-AT + Csp1(1-100)
NF-AT + Csp1(101-197)
iono.

Figure 6

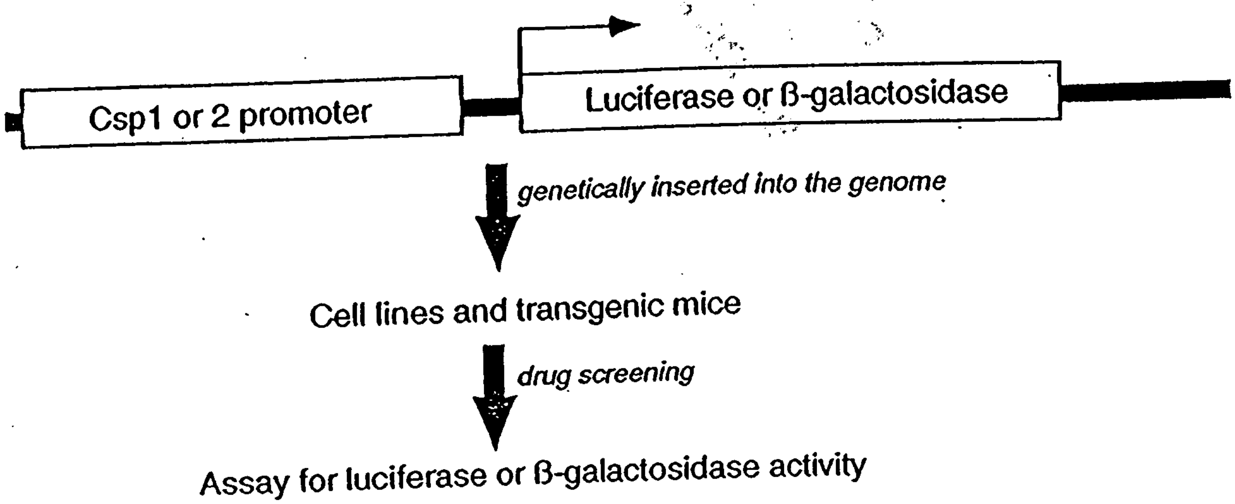

Csp1 or 2 promoter
Luciferase or ß-galactosidase
genetically inserted into the genome
Cell lines and transgenic mice
drug screening
Assay for luciferase or ß-galactosidase activity

Figure 7

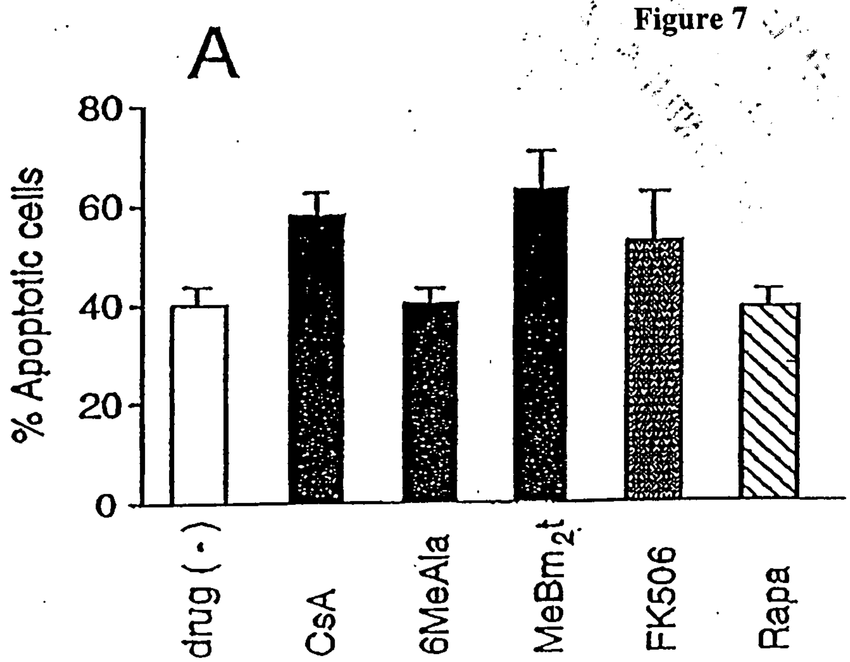

A
% Apoptotic cells
80
60
40
20
0
drug (-)
CsA
6MeAla
MeBm2t
FK506
Rapa

Figure 8

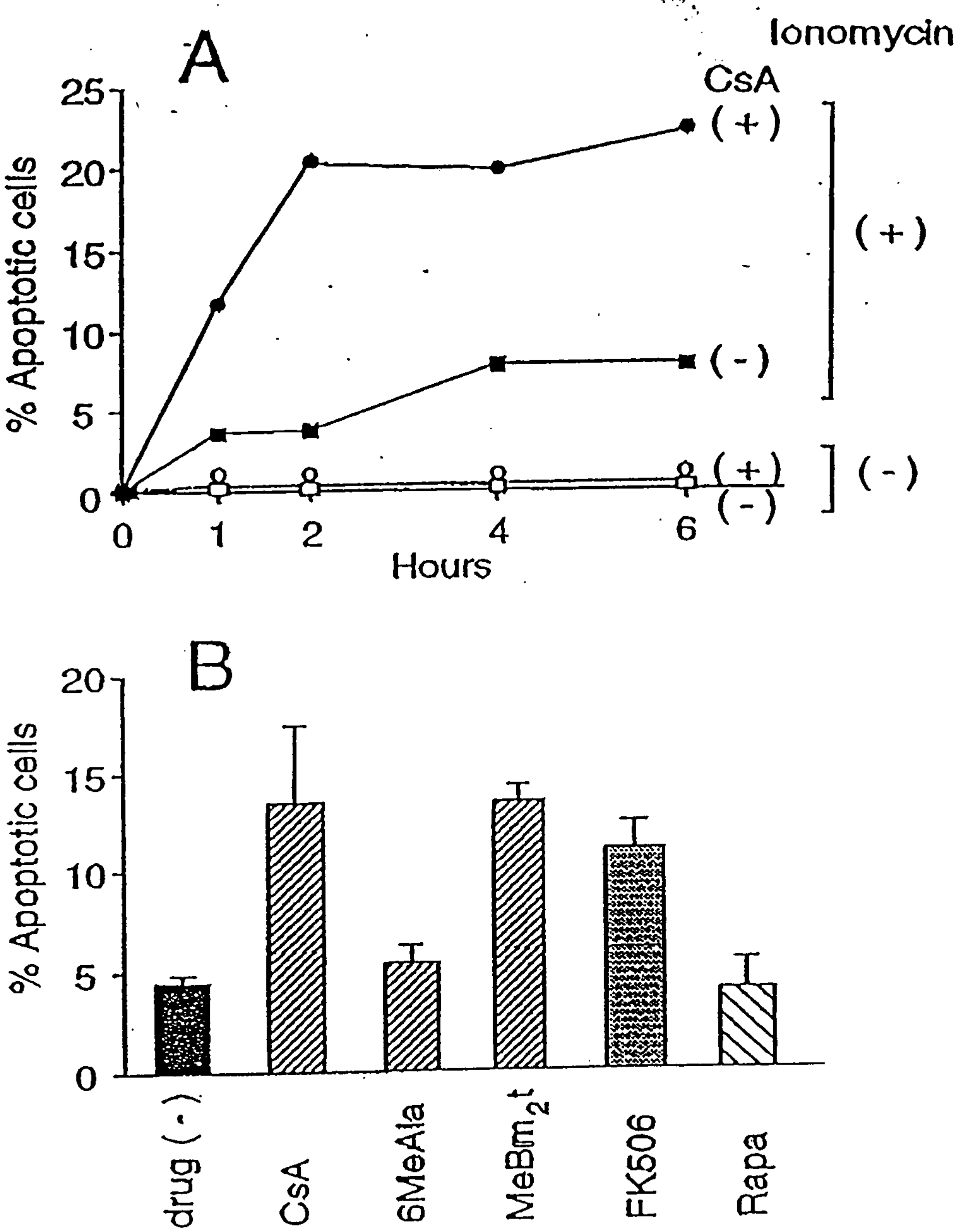

A
Ionomycin
CsA
(+)
(+)
(-)
(+)
(-)
(-)
% Apoptotic cells
0
5
10
15
20
25
0
1
2
4
6
Hours
B
% Apoptotic cells
0
5
10
15
20
drug (-)
CsA
6MeAla
MeBm$_2$t
FK506
Rapa

Figure 9

human Csp1 promoter (2.5kb) (SEQ ID NO: 1)

```
   1 cttgggttta gctccctgag gacacaaact gtcctaagac tatgataata
     gtaatcatag aaccgtgcac atggcaagtt ctgaataaat ctcagctgtt  100  MyoD
 101 ggatatactt tttgttataa ttactaacac ttcctaacta gagagtaagc
     ctactctaag aaaaaatata actgtaattt cacaacctcc aaagaaccca  200
 201 gtgcataaac agctaccatt tattaagcac tgactgaatt cttagtaata
     tgtcttcatt tttttcagat gaggaaacta agattcagct tatttgtaca  300  MyoD, NF-AT
 301 agtagttaaa aagcaaagct gaaattcaga cccaagttct cactgtatca
     tactgtccaa aaaagaattc tatttttcag gaagagacat gtctgctcac  400
 401 ttgaggtcct cttatttttc cgctattccc caaaggaaag gggtgatctc       NF-AT,NF-AT
     ttaattcttt cgttatgtcc tattgtacat agcatataat ggtaattcag  500
 501 aaaaattact tctaattaca taaattttca caatggtata gtgactaata
     cgctgaaata gaaaagtaag gcattgttat catggtctag ttcagtcttt  600  NF-AT
 601 attgcgacta tatctgataa tatacggtaa gcatctaacc acttgccagg
     ggccacagag ccacagggag actatgtctc gcttaaattc ccaaaagtgg  700
 701 gcccctgtgc ttcaaaacgt ccccgcatgg gaaccacaaa aacgttgcct
     ccccagttat caccccaagg gcccaagagc cgaggactct gcccggcgtc  800
 801 cttcagctgg caccagctgt cagaaaagcg gaactgggga cgaggacttt       MyoD
     gcccctaacc aacatggccg ccctgaggct tcgggcttcg ggcggcagaa  900
 901 ggaaggtcac gtgaagagaa ttccgttcct ttattggccc cgtctcctgg       MyoD
     aagggcgggg tacaataacc caaccggcgc cggccttaaa ggggccaccg 1000
1001 ttggatctgc cggtggccgg ccctaggggc tggggggcg gtcgccgcgc
     cgggcttctg cccctcccgc gcggaacggt gacgggcggg gctggcgctg 1100
1101 ggaggccgtg tcgctgggag actgctgaca gcccgccgcc tgccgccgcg
     cgattccgag ggggttaacg gcggagccgc cggccgggcg cggaccggag 1200
1201 cgcgtgaggc tccggcgcgc aagcccggag cagcccgctg gggcgcacag
     ggtcgcgcgg gcgcggggat ggaggacggc gtggccggtc cccagctcgg 1300
1301 ggccgcggcg gaggcggcgg aggcggccga ggcgcgagcg cggcccgggg
     tgacgctgcg gcccttcgcg cccctctcgg gggcggccga ggcggacgag 1400
1401 ggcggcggcg actggagctt cattgactgc gagatggagg aggtggacct
     gcaggacctg cccagcgcca ccatcgcctg tcacctggac ccgcgcgtgt 1500 MyoD
1501 tcgtggacgg cctgtgccgg gtgaggaccg cgccgggcgg gccgtcgggg
     cggagggcgg acacttgttg cccgaggagg cggcgcgggt cgcagcgccc 1600 MyoD
1601 agtcccggcc gcgcgcgggg cggggaggca gcgacgtccc ccgggctgct
     cggccgcgga cccgtcaggg ctggggcgtg gggacggcgc cccgaggtc  1700
1701 ccggtcccct agcacccccg gggcgcgcgg agctcactgc agagtcccac
     aggctcgccc cggcccccgt gtgcgcccag gctggtgcga ctaggggggt 1800
1801 gaattcgctc cccaaggtgg ggcagcgccg ccgcccccctg cgctctcgcc
     atcgccccgc atttactcgc tggaggaggg ggtcacctca ttcctaggga 1900
1901 ggaggaaaca gacattgagc ggcgacgtga ctcagtgttc ataaatagga       NF-AT, TATA
     cgacgtccct gcattcccaa tctgcactat tggaagaaaa gccaatgttt 2000
2001 gggtgaggat ccgtggttgc tcattagcca gcggctggcc agttttggtg
     gaattgtgtt gggggaaggg ggaccatctt tcagaccttt aggatattta 2100
2101 gtcaagaacc ttgcccccctt gtgtgaaggt gtggcttgcc gccatcgggg
     acacccagta catggggagt cgactccttc cccgcctcc ccccaccccc 2200
2201 gcaaaatcca cacaatttag acactttgga gggtgagggg caggtatgag
     taatcaataa tggtggtggg gaggaagaat ttatttcaaa tctgcagtta 2300
2301 ttgtgcagaa taaaatgtgg acaacgtggg cgtcacagaa tgaaaccggt
     ctttgagaga tgccccatta ggagagcagc tgtcaaaaaa agcagtgctt 2400
2401 tcagcgcttg gctgtgggtc cacaaatgct gtcaatgaac tatagttgaa
     ggctgctgcc aatacaacac cactgtgaaa caga                   2484
```

Figure 10

murine Csp1 (SEQ ID NO: 2)

```
1                                                 31
ATG GAG GAG GTG GAT CTG CAG GAC CTG CCG AGC GCC ACC ATC GCC TGC CAC CTG GAC CCG
61                                                91
CGC GTG TTC GTG GAC GGC CTG TGC CGG GCC AAA TTT GAA TCC CTC TTC AGA ACA TAT GAC
121                                               151
AAG GAC ACC ACC TTC CAG TAT TTT AAG AGC TTC AAA CGT GTC CGG ATA AAC TTC AGC AAC
181                                               211
CCC TTA TCT GCA GCC GAT GCC AGG CTG CGG CTG CAC AAG ACC GAG TTC CTG GGG AAG GAA
241                                               271
ATG AAG TTG TAT TTT GCT CAG ACT TTA CAC ATA GGA AGT TCA CAC CTG GCT CCG CCC AAT
301                                               331
CCC GAC AAA CAG TTC CTC ATC TCC CCT CCG GCC TCT CCT CCC GTT GGC TGG AAA CAA GTA
361                                               391
GAA GAT GCC ACC CCC GTC ATA AAT TAC GAT CTT TTA TAT GCC ATC TCC AAG CTG GGG CCA
421                                               451
GGA GAG AAG TAT GAA CTG CAT GCA GCG ACA GAC ACC ACT CCC AGT GTG GTG GTC CAC GTG
481                                               511
TGT GAG AGT GAC CAA GAG AAT GAG GAG GAA GAG GAA GAG ATG GAG AGA ATG AAG AGA CCC
541                                               571
AAG CCC AAA ATC ATC CAG ACA CGG AGA CCG GAG TAC ACA CCC ATC CAC CTC AGC TGA
```

coding sequence: 597 nucleotides

Figure 11

murine Csp2 (SEQ ID NO: 3)

1 / 31
GAA TTC GTC GAC CCA CGC GTC CGC CCA CGC GTC CGC TTG GGG CAG CAG GCA TCT ATC CCT

61 / 91
GAA GAT GGG GGA CTT TTC TTC CTC TGC TGC ATA GAC AGA GAC TGG GCT GTC ACT CAG TGT

121 / 151
TTT GCT GAA GAG GCC TTC CAA GCA CTC ACT GAC TTC AGT GAT CTC CCC AAC TCA TTG TTT

181 / 211
GCC TGC AAT GTT CAC CAG TCT GTG TTT GAA GAA GAG GAG AGC AAG GAA AAA TTC GAG GGA

241 / 271
CTG TTC CGG ACC TAT GAT GAA TGT GTG ACG TTC CAG CTG TTT AAG AGT TTC CGA CGG GTT

301 / 331
CGA ATA AAT TTC AGC CAT CCC AAA TCT GCA GCC CGT GCC CGG ATA GAG CTT CAT GAG ACT

361 / 391
CAG TTC AGA GGG AAG AAG CTA AAA CTC TAC TTC GCC CAG GTC CAG ACC CCA GAG ACA GAT

421 / 451
GGA GAC AAA CTG CAT TTG GCA CCT CCA CAG CCT GCC AAA CAG TTC CTC ATC TCA CCC CCT

481 / 511
TCA TCT CCA TCT GTT GGC TGG AAG CCT ATC AGC GAT GCC ACA CCA GTC CTC AAC TAT GAC

541 / 571
CTT CTT TAT GCT GTG GCC AAA CTA GGA CCA GGA GAG AAA TAT GAG CTG CAC GCT GGA ACT

601 / 631
GAG TCT ACC CCG AGC GTC GTG GTG CAT GTG TGT GAC AGC GAC ATG GAG AGG GAG GAG GAC

661 / 691
CCA AAG ACT TCC CCA AAG CCA AAA ATC AAT CAG ACC CGG CGG CCT GGC CTG CCA CCC TTC

721
GGT CAC TGA

coding sequence: 729 nucleotides

Figure 12

murine Csp1 (SEQ ID NO: 4)

```
1/1                                     31/11
ATG GAG GAG GTG GAT CTG CAG GAC CTG CCG AGC GCC ACC ATC GCC TGC CAC CTG GAC CCG
M   E   E   V   D   L   Q   D   L   P   S   A   T   I   A   C   H   L   D   P
61/21                                   91/31
CGC GTG TTC GTG GAC GGC CTG TGC CGG GCC AAA TTT GAA TCC CTC TTC AGA ACA TAT GAC
R   V   F   V   D   G   L   C   R   A   K   F   E   S   L   F   R   T   Y   D
121/41                                  151/51
AAG GAC ACC ACC TTC CAG TAT TTT AAG AGC TTC AAA CGT GTC CGG ATA AAC TTC AGC AAC
K   D   T   T   F   Q   Y   F   K   S   F   K   R   V   R   I   N   F   S   N
181/61                                  211/71
CCC TTA TCT GCA GCC GAT GCC AGG CTG CGG CTG CAC AAG ACC GAG TTC CTG GGG AAG GAA
P   L   S   A   A   D   A   R   L   R   L   H   K   T   E   F   L   G   K   E
241/81                                  271/91
ATG AAG TTG TAT TTT GCT CAG ACT TTA CAC ATA GGA AGT TCA CAC CTG GCT CCG CCC AAT
M   K   L   Y   F   A   Q   T   L   H   I   G   S   S   H   L   A   P   P   N
301/101                                 331/111
CCC GAC AAA CAG TTC CTC ATC TCC CCT CCG GCC TCT CCT CCC GTT GGC TGG AAA CAA GTA
P   D   K   Q   F   L   I   S   P   P   A   S   P   P   V   G   W   K   Q   V
361/121                                 391/131
GAA GAT GCC ACC CCC GTC ATA AAT TAC GAT CTT TTA TAT GCC ATC TCC AAG CTG GGG CCA
E   D   A   T   P   V   I   N   Y   D   L   L   Y   A   I   S   K   L   G   P
421/141                                 451/151
GGA GAG AAG TAT GAA CTG CAT GCA GCG ACA GAC ACC ACT CCC AGT GTG GTG GTC CAC GTG
G   E   K   Y   E   L   H   A   A   T   D   T   T   P   S   V   V   V   H   V
481/161                                 511/171
TGT GAG AGT GAC CAA GAG AAT GAG GAG GAA GAG GAA GAG ATG GAG AGA ATG AAG AGA CCC
C   E   S   D   Q   E   N   E   E   E   E   E   E   M   E   R   M   K   R   P
541/181                                 571/191
AAG CCC AAA ATC ATC CAG ACA CGG AGA CCG GAG TAC ACA CCC ATC CAC CTC AGC TGA
K   P   K   I   I   Q   T   R   R   P   E   Y   T   P   I   H   L   S   *
```

198 amino acids and 597 nucleotides

Figure 13

murine Csp2 (SEQ ID NO: 5)

1/1 31/11
GAA TTC GTC GAC CCA CGC GTC CGC CCA CGC GTC CGC TTG GGG CAG CAG GCA TCT ATC CCT
E F V D P R V R P R V R L G Q Q A S I P

61/21 91/31
GAA GAT GGG GGA CTT TTC TTC CTC TGC TGC ATA GAC AGA GAC TGG GCT GTC ACT CAG TGT
E D G G L F F L C C I D R D W A V T Q C

121/41 151/51
TTT GCT GAA GAG GCC TTC CAA GCA CTC ACT GAC TTC AGT GAT CTC CCC AAC TCA TTG TTT
F A E E A F Q A L T D F S D L P N S L F

181/61 211/71
GCC TGC AAT GTT CAC CAG TCT GTG TTT GAA GAA GAG GAG AGC AAG GAA AAA TTC GAG GGA
A C N V H Q S V F E E E E S K E K F E G

241/81 271/91
CTG TTC CGG ACC TAT GAT GAA TGT GTG ACG TTC CAG CTG TTT AAG AGT TTC CGA CGG GTT
L F R T Y D E C V T F Q L F K S F R R V

301/101 331/111
CGA ATA AAT TTC AGC CAT CCC AAA TCT GCA GCC CGT GCC CGG ATA GAG CTT CAT GAG ACT
R I N F S H P K S A A R A R I E L H E T

361/121 391/131
CAG TTC AGA GGG AAG AAG CTA AAA CTC TAC TTC GCC CAG GTC CAG ACC CCA GAG ACA GAT
Q F R G K K L K L Y F A Q V Q T P E T D

421/141 451/151
GGA GAC AAA CTG CAT TTG GCA CCT CCA CAG CCT GCC AAA CAG TTC CTC ATC TCA CCC CCT
G D K L H L A P P Q P A K Q F L I S P P

481/161 511/171
TCA TCT CCA TCT GTT GGC TGG AAG CCT ATC AGC GAT GCC ACA CCA GTC CTC AAC TAT GAC
S S P S V G W K P I S D A T P V L N Y D

541/181 571/191
CTT CTT TAT GCT GTG GCC AAA CTA GGA CCA GGA GAG AAA TAT GAG CTG CAC GCT GGA ACT
L L Y A V A K L G P G E K Y E L H A G T

601/201 631/211
GAG TCT ACC CCG AGC GTC GTG GTG CAT GTG TGT GAC AGC GAC ATG GAG AGG GAG GAG GAC
E S T P S V V V H V C D S D M E R E E D

661/221 691/231
CCA AAG ACT TCC CCA AAG CCA AAA ATC AAT CAG ACC CGG CGG CCT GGC CTG CCA CCC TTC
P K T S P K P K I N Q T R R P G L P P F

721/241
GGT CAC TGA
G H *

242 amino acids and 729 nucleotides

Figure 14

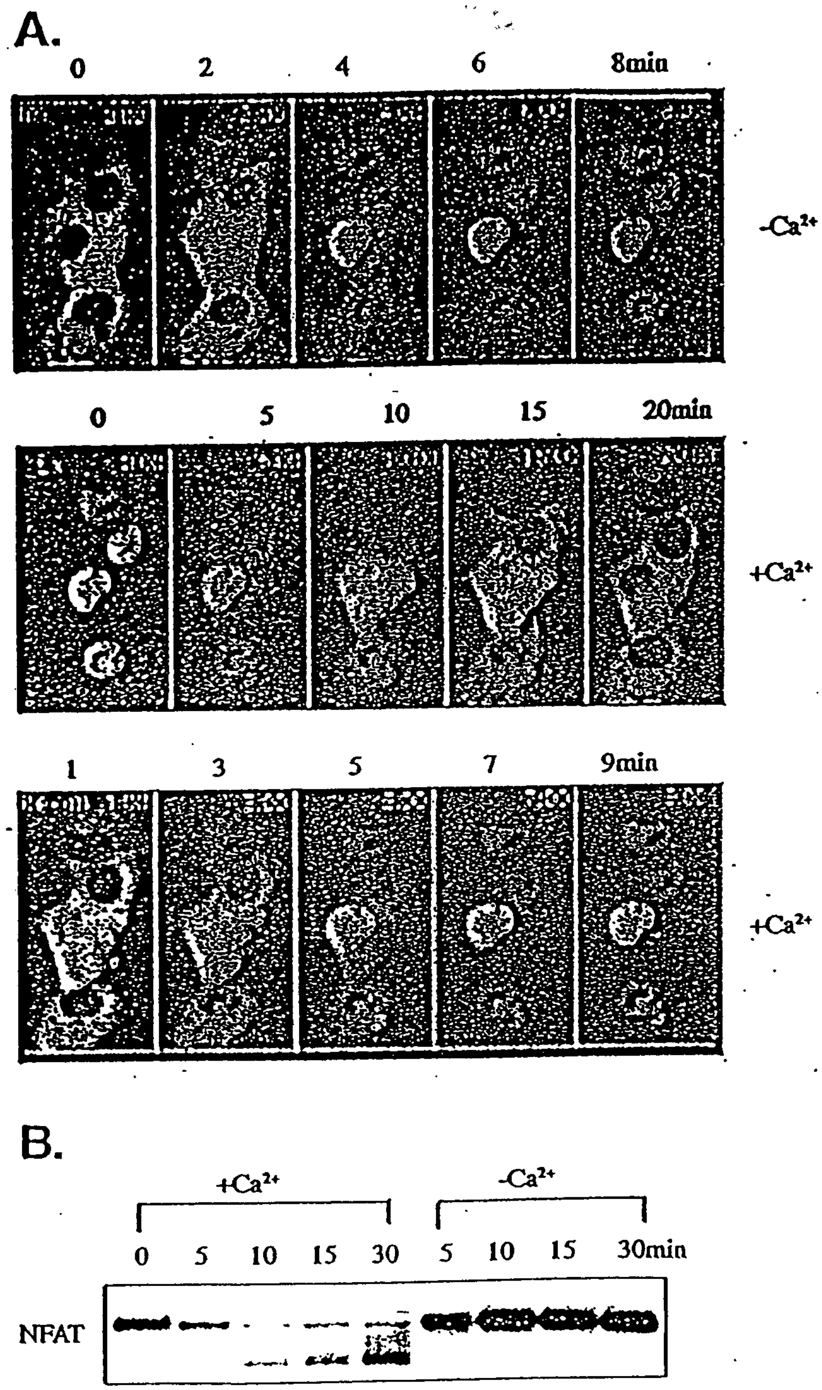

A.
0
2
4
6
8min
-Ca²⁺
0
5
10
15
20min
+Ca²⁺
1
3
5
7
9min
+Ca²⁺
B.
+Ca²⁺
-Ca²⁺
0
5
10
15
30
5
10
15
30min
NFAT

Figure 15

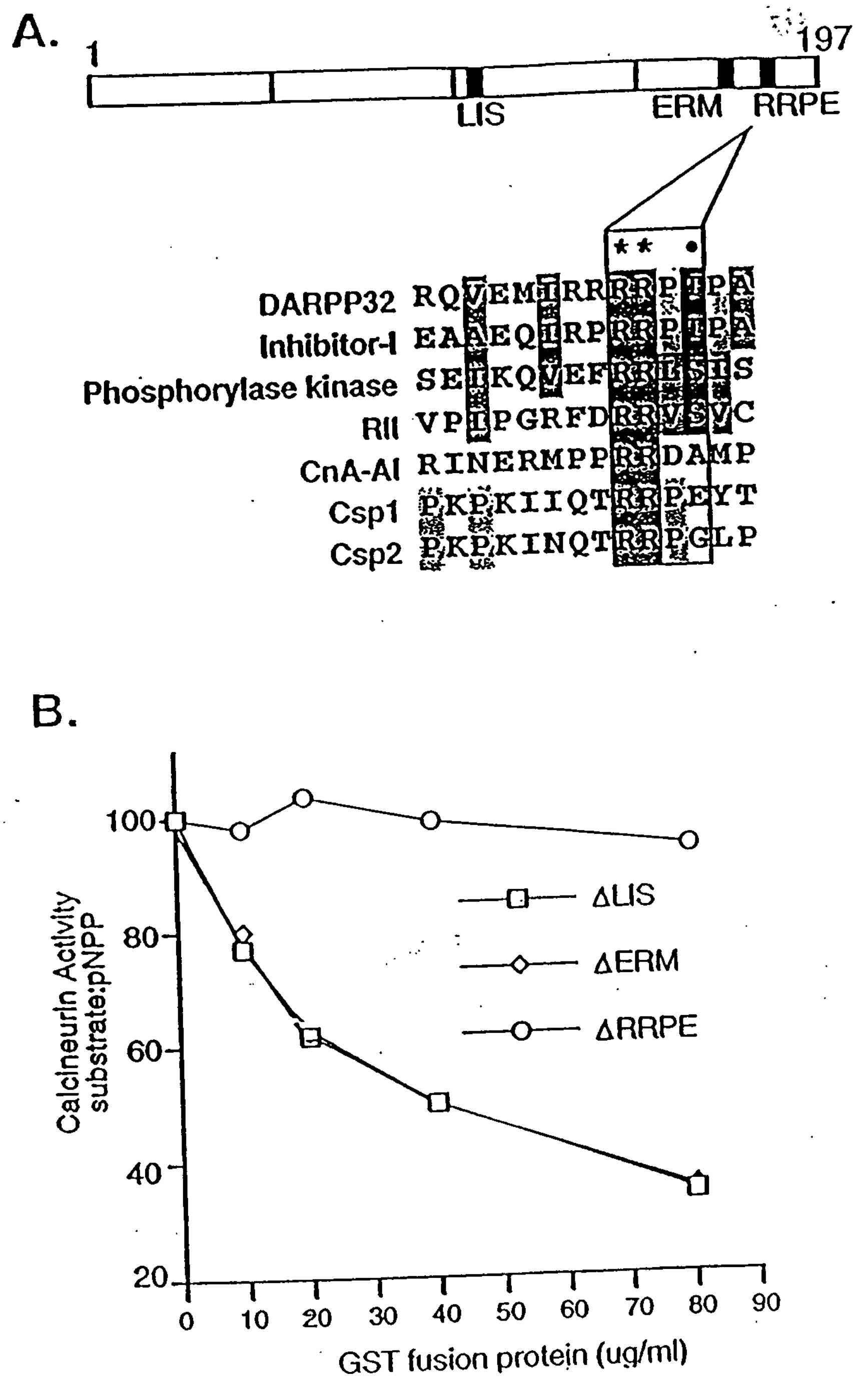

A.
1
197
LIS
ERM
RRPE
DARPP32 RQVEMIRRRRPTPA
Inhibitor-I EAAEQIRPRRPTPA
Phosphorylase kinase SEIKQVEFRRLSIS
RII VPIPGRFDRRVSVC
CnA-AI RINERMPPRRDAMP
Csp1 PKPKIIQTRRPEYT
Csp2 PKPKINQTRRPGLP
B.
Calcineurin Activity substrate:pNPP
100
80
60
40
20
ΔLIS
ΔERM
ΔRRPE
0
10
20
30
40
50
60
70
80
90
GST fusion protein (ug/ml)

Figure 16

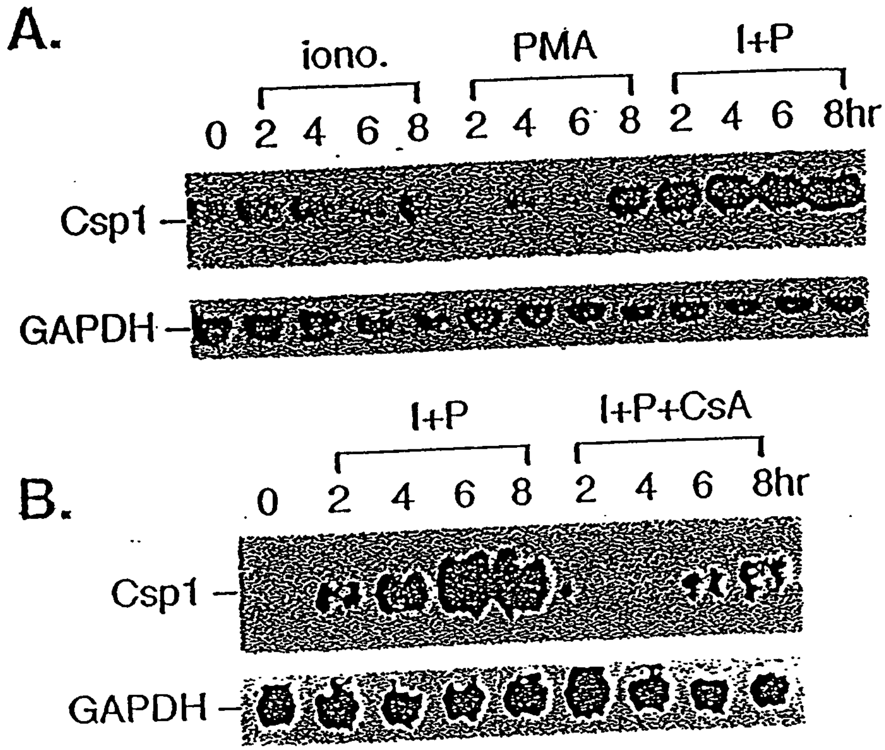

A.
iono.
PMA
I+P
0 2 4 6 8 2 4 6 8 2 4 6 8hr
Csp1
GAPDH
B.
I+P
I+P+CsA
0 2 4 6 8 2 4 6 8hr
Csp1
GAPDH

Figure 17

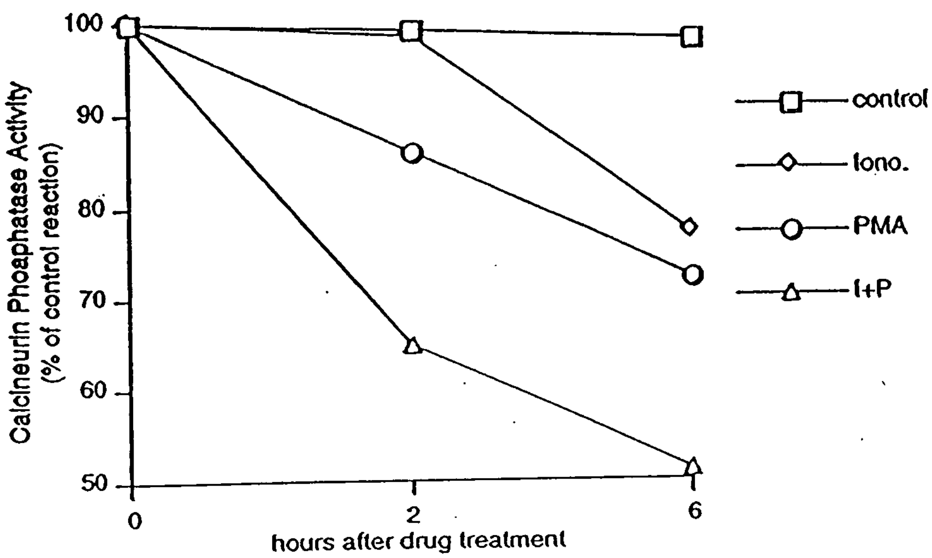

100
90
80
70
60
50
Calcineurin Phosphatase Activity
(% of control reaction)
0
2
6
hours after drug treatment
control
Iono.
PMA
I+P

Figure 18

Murine Csp3 (SEQ ID No: 22)
cDNA Nucleic acid sequence (coding)

atgctccgagacagcctgaaatcttggaatgacagccagtcagacctctgtagcagcgaccaggaggaggaagaggagatggtcttcggt
gaaaatgaggacggactggaagagatgatggacctaagtgacctgcccacctcactctttgcttgcagtgtccatgaagcagtgtttgaggt
ccaagagcaaaaggagaggtttgaggccctgttcaccctctacgatgaccaggtcacattccagttgttcaagagttttcgcagagtgaggat
caacttcagcaagcccgcaagagcgcggatagagctccacgagagtgagttccacggacggaagctgaagctttacttcgcacaggtgca
ggtgtccggggaggcccgggacaagtcctacttactgccaccacaacccaccaagcagttcctcatctcccctcccgcttcatcccccgtgg
ggtggaagcagagtgaagatgcgatgccagtgatcaactatgacctgctctgcgctgtctccaagctgggcccaggggagaaatacgaac
tgcacgcgggaaccgagtccacccccagtgtggtggtgcacgtctgtgagagcgaaactgaagaggaagaagacacaaaaaatccaaaa
cagaaaatcacgcagacgcggcgcccggaggctcccacggcggcactgagtgagcggctggactgtgcactctga

Figure 19

cDNA nucleic acid sequence
(entire coding + 5' and 3' UTR) (SEQ ID No: 23)

gccgctgcggcccgcgttgagggcgtggtggctccgggtggtgagggtctgtccgccccaggccgcgctcgtggg
catccccccctcgggcctctcccctcgagcgcacagaagtatctggcaggcatcctagaactttacagagaagatgctc
cgagacagcctgaaatcttggaatgacagccagtcagacctctgtagcagcgaccaggaggaggaagaggagatg
gtcttcggtgaaaatgaggacggactggaagagatgatggacctaagtgacctgcccacctcactctttgcttgcagtg
tccatgaagcagtgtttgaggtccaagagcaaaaggagaggtttgaggccctgttcaccctctacgatgaccaggtca
cattccagttgttcaagagttttcgcagagtgaggatcaacttcagcaagcccgcaagagcgcggatagagctccacg
agagtgagttccacggacggaagctgaagctttacttcgcacaggtgcaggtgtccggggaggcccgggacaagtc
ctacttactgccaccacaacccaccaagcagttcctcatctcccctcccgcttcatcccccgtggggtggaagcagagt
gaagatgcgatgccagtgatcaactatgacctgctctgcgctgtctccaagctgggcccaggggagaaatacgaact
gcacgcgggaaccgagtccacccccagtgtggtggtgcacgtctgtgagagcgaaactgaagaggaagaagacac
aaaaaatccaaaacagaaaatcacgcagacgcggcgcccggaggctcccacggcggcactgagtgagcggctgg
actgtgcactctgagcggctgcggtgcctgccgcgcctgcctgtcccaccactacagctgcgcctgtctaggagcaca
gcccagggatgctcttgcatccgtcag

Figure 20

Murine Csp3 (SEQ ID NO: 24)
Amino acid sequence

MLRDSLKSWNDSQSDLCSSDQEEEEEMVFGENEDGLEEMMDLSDLPTSLFACSVHEAV
FEVQEQKERFEALFTLYDDQVTFQLFKSFRRVRINFSKPARARIELHESEFHGRKLKLYF
AQVQVSGEARDKSYLLPPQPTKQFLISPPASSPVGWKQSEDAMPVINYDLLCAVSKLGP
GEKYELHAGTESTPSVVVHVCESETEEEEDTKNPKQKITQTRRPEAPTAALSERLDCALZ

Figure 21

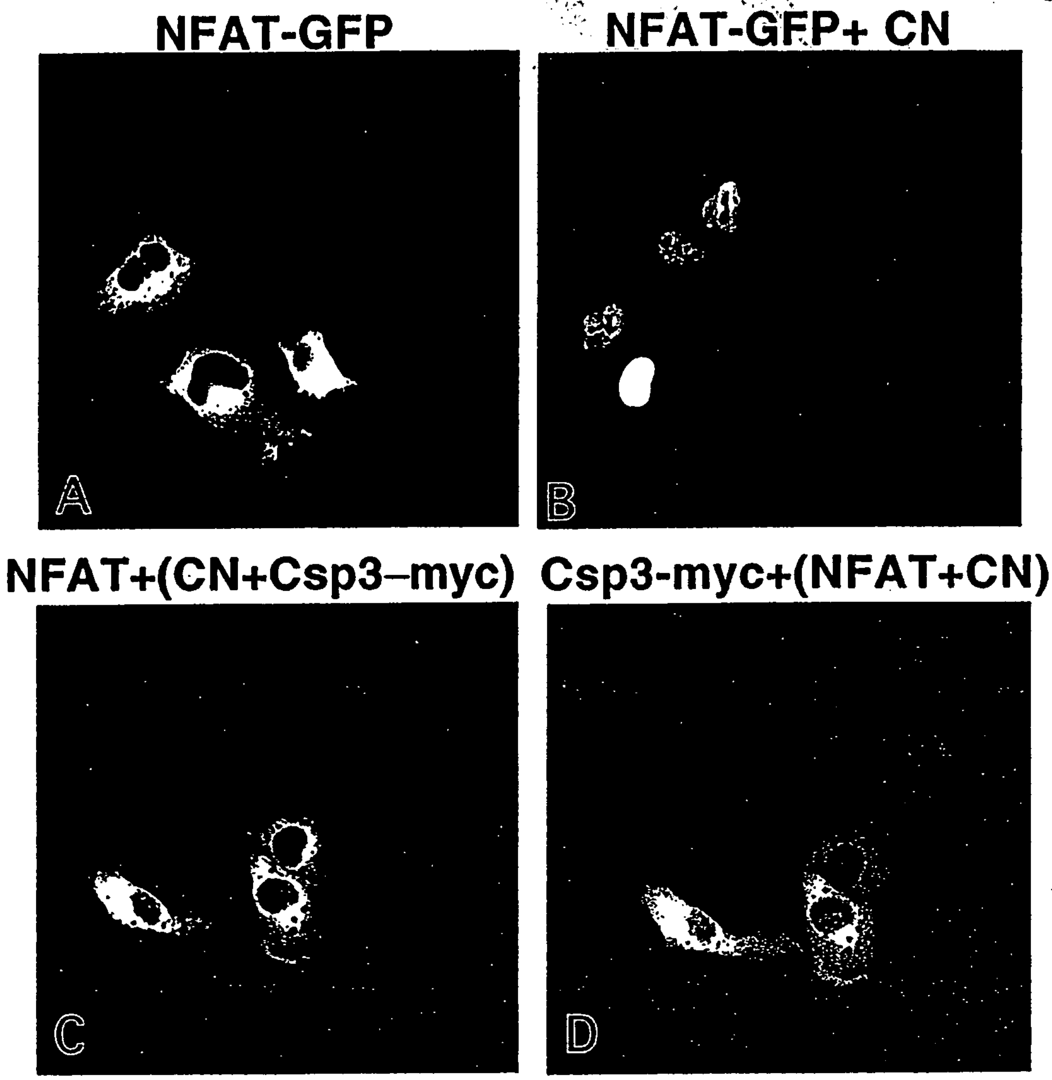
Identification of a Third Calcipressin Family Member, Csp3

```
csp2    1 ----------------------------------------MDCDVSTLVACVVDVEVFT
csp3    1 MLRDSLKSWNDSQSDLCSSDQEEEEEMVFGENEDGLEEMMDLSDLPTSLFACSVHEAVFE
csp1    1 ------------------------------------MEEVDLQDLPSATIACHLDPRVFV

csp2   20 NQEVKEKFEGLFRTYDECVTFQLFKSFRRVRINFSNPKSAARARIELHETQFRGKKLKLY
csp3   61 VQEQKERFEALFTLYDDQVTFQLFKSFRRVRINFSKP---ARARIELHESEFHGRKLKLY
csp1   25 DGLCRAKFESLFRTYDKDTTFQYFKSFKRVRINFSNPLSAADARLRLHKTEFLGKEMKLY

csp2   80 FAQVQTPETDGDKLHLAPPQPAKQFLISPPSSPSVGVKPISDATPVLNYDLLYAVAKLGP
csp3  118 FAQVQVSGEARDKSYLLPPQPTKQFLISPPASSPVGWKQSEDAMPVINYDLLCAVSKLGP
csp1   85 FAQTLHIGS----SHLAPPNPDKQFLISPPASPPVGWKQVEDATPVINYDLLYAISKLGP

csp2  140 GEKYELHAGTESTPSVVVHVCDSDMEREEDPKTS------PKPKINQTRRPGLPPFVSN--
csp3  178 GEKYELHAGTESTPSVVVHVCESETEEEEDTKN-------PKQKITQTRRPEAPTAALSER
csp1  141 GEKYELHAATDTTPSVVVHVCESDQEKEEEEEMERMKRPKPKIIQTRRPEYTPIHLS--

csp2      -----
csp3  232 LDCAL
csp1      -----
```

A third calcipressin family member, termed csp3, was cloned from murine T cells and found to have high sequence homology with csp1 and csp2.

Calcipressin 3 Inhibits Calcineurin Mediated Translocation of NFAT

Figure 22



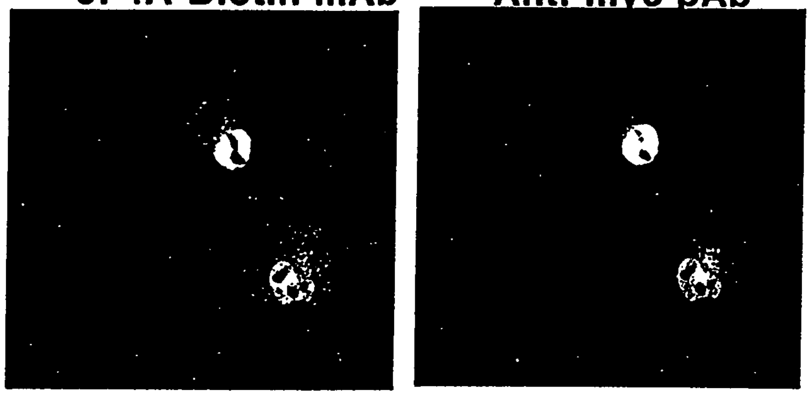
Panel A demonstrates the cytoplasmic expression pattern of the transcription factor NFAT tagged with green fluorescent protein (GFP) in the absence of stimulus. Upon co-expression of calcineurin (CN), NFAT shuttles into the nucleus as seen in panel B.

Panel C demonstrates the cytoplasmic expression of NFAT in the presence of calcineurin and calcipressin 3 (Csp3), suggesting inhibition of CN activity by Csp3. Csp3 co-expression is demonstrated in panel D by immunostaining with an anti-myc antibody to detect the myc-tagged Csp3 protein.

Generation of anti-Csp2 and anti-Csp1 Monoclonal Antibodies

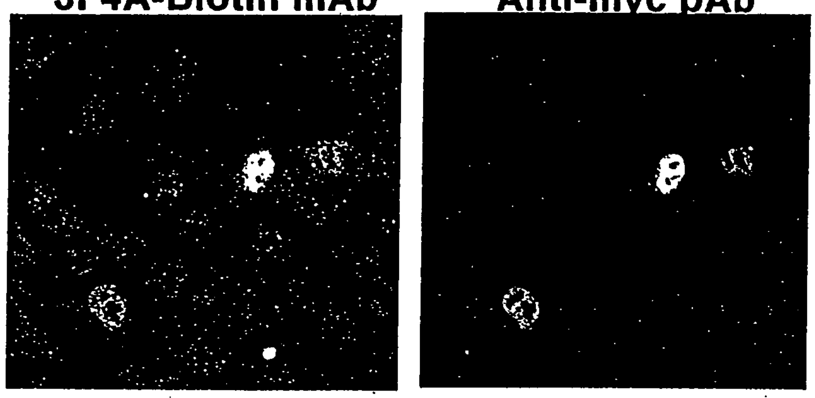
Figure 23

3F4A-Biotin mAb | Anti-myc pAb



Myc-Csp2 transfected BHKcells

3F4A-Biotin mAb | Anti-myc pAb



Myc-Csp1 transfected BHK cells

Monoclonal antibodies (mAb) were generated against Csp1 and Csp2. 3F4A mAb was biotinylated and demonstrated to recognize cells transfected with both myc-tagged csp2 (top panel) and csp1 (bottom panel), as verified by immunostaining with a myc pAb.

Figure 24A

```
        10        20        30        40        50        60        70
GCCAAATTTGAATCCCTCTTCAGAACATATGACAAGGACACCACCTTCCAGTATTTTAAGAGCTTCAAAC 70
GTGTCCGGATAAACTTCAGCAACCCCTTATCTGCAGCCGATGCCAGGCTGCGGCTGCACAAGACCGAGTT 140
CCTGGGGAAGGAAATGAAGTTGTATTTTGCTCAGGTAAGTGTGTTCATTGTGAAGCGGGTTCCTCCCGGC 210
AAAGCACCTTATACATTGGAAACCTAGAGGTCACCTCAAAACAGACAGGATTCCAACCTTGAGTTCTTAA 280
GGTCTCCCTGCTGTGTAAAGGGATCTGGTGAAGGGGACAGTAAGCCTGGACCTTCCTGGGTTAAACCGTG 350
       360       370       380       390       400       410       420
AAGGAAGGAGAGCAAGCTTCCCTTGGTCACCAGAAAGCTTAGGGATTTGGAGGGGAGAAGAGGGCATCGC 420
TGCCCCCCTCCCTGCACACTAGTCAGCTTCACTGGGACTAGGCCAGCGACCTGTCAAGAGCTGTCTCAAG 490
CCAGTGCAGGTTCTCCACGCCTCACCTTGTAAGCCTGTATTCAGATCAGCACAGGGCTGTCAGTCGGGGC 560
AGGGGTGAGGGTCATCACATGGTTGAGACTCTTAGCTGAGGGGCAGAAAAGGGGGCTGTGGATGAGTTGT 630
CCATTGTTCTGCCAACCTCGGGGACACCTTCAAGGCAGCTCCCAACTTCCATGTGACTGTAACGGGGACT 700
       710       720       730       740       750       760       770
GGTAGATCGCAGCTTCTCGTTGTTATCCCCAAGGTAATGTCAGTCCTTGCCAGGCTCTGAAGCCGCTTCC 770
TTTCTTCTCAGTTGTCTACACTCACTTCCTGCCAGCTTAGGGCCAGCGGAGTCCTGTGGAGTGTGGCTCA 840
TGGCCCTCACCTCTCGGTAATGGTAGATTTTGACCATGAAATACCCTCTGTGGCTCATGTATTTGAATAC 910
TTGGGTCCTCTGTGGTGCAGTTTTACAGTTAGGGAACTTTAGGAGGTGGGGCCTCCCTAAAGGAATGAGA 980
TCCCCGAGGCAGACTCTGAGGGGTTAGAGCCCAGCCCCTTGTCAGATTGAAGCTCTTTGCTTCCTGGTTG 1050
      1060      1070      1080      1090      1100      1110      1120
GCACCATGTAACAGGTTACCACAGGCTTCTGCAGCCTCTAGCTACCATGACATCCGTCTTTTCTGCCTTC 1120
CCTATGATGGCTGCGCACTCTCGAACTGTGAGCCAGGATAAGGCCTTCCCGCTTTGGTTTTCATCCAGGG 1190
CTGTCATAGACACTTGAAAAGTTTACCCAACACAGGCACCAAATCCGGAATTCAGTCCTTCCTTCACCTC 1260
TATACAGACCACATTTCTGCTTCTTGGAATCGTACCTGGTCCAGAGCCTGACCATCGGTCTGCCCTTCCA 1330
TGCTTGCCTTCCAGAAGCTTCCATGAACTGTCGTGACCTCGCTCGCTTGCTGCATAATGATGAACTCATT 1400
      1410      1420      1430      1440      1450      1460      1470
TCTCTCCTCAGACTTTACACATAGGAAGTTCACACCTGGCTCCGCCCAATCCCGACAAACAGTTCCTCAT 1470
CTCCCCTCCGGCCTCTCCTCCCGTTGGCTGGAAACAAGTAGAAGATGCCACCCCCGTCATAAATTACGAT 1540
CTTTTATATGCCATCTCCAAGCTGGGGCCAGGTAAGCAGCACCCTCAGGTGGGAAAGTGTCGGGAGGTGT 1610
GGAGAGACTCTCTGGGGTCCCCAGGCCTCACGCGCCCCCATGCTGTCGTATGGTGTGACCCCTGCGTTAT 1680
TCCACATTGCTGCAGCTCGTGCTGGAGTGTGTGCCCCTTGGAGGATTCCAGGAGATGGTAGCAACCTGTG 1750
      1760      1770      1780      1790      1800      1810      1820
GGTTTGTGCACCACTGTCCCCCCCCAAGTGTCCCCCGAATCTATCCCTTCACCCAGCAGGCACACCTGTG 1820
TGGCTCACTCCAGGCCCCAGATCATGTTGTTCCAGGTGGGATGGGAAAGGGCAAACAGACCAACCTCTAG 1890
GGAGTCTCGTCAACTGTCATTCCTACTTCCGTACTGGGTGGGAGGGATGTGCGCATCTCTCACCCCACAC 1960
AGCAAGCCGAATCAGCACTGCCCATCAGCCCCTCGTCATCTGAAGTTCCTTTAGGGCAAGGGTTTTATTT 2030
TCATGGCTCATCAGCAGAAAGATTACATTTCTGAGAACACAGCCTAAATGGAAATTCCTCCCGCGGTACA 2100
```

Figure 24B

```
      2110      2120      2130      2140      2150      2160      2170
AACTGAGACTCACGTTACTAGTGCTAATTGTAGCATGAAGGTCAAAAGTGGAAACGGCCAGTGTGAGCAA 2170
GGAGACGGCTCAGCATGGCGGCTCTCAGCACAGTTGAGGGGTCTGTTGTCTGTGGATGTGTTATACATGG 2240
ACACAGACCTCCATCTGCCGCAAGGGAACAGGCTGTTCCAGAGGCAGGAATTGAGGCGAGCCTTCTGTCT 2310
TTAAGAACCCAAACCAGAAATGAAGGGGCTGAAACATTCCTACCAGGGCCATGACAGAGTTCTCCACACC 2380
CAGAGCCAGCACACTTCAGTCAGCCTTCGGGGCTGCAAAGGCGGCTTGTGGAGAGCAGTCTGACCTTCAT 2450
      2460      2470      2480      2490      2500      2510      2520
CCACGAAGTTAGTGCTGTGTGTGTCTGTGCGTGCCCGCAGCTCTCTACCTTTGGGCCAAGGGTAGATAGG 2520
TATAGAAACGCCCCCTCCACTTACAGTTTTCCCAGCAGCCCTCAACACTTGGGGAGAGCCGAGCTCCTTC 2590
GTTTTTTTAGCCTCATTGGTGGGGTAGAGAGGCCATGCTGCCTCGTTGTTCATGAGTTCTGTGCCTCCCA 2660
CATCTATGGAGCAGACTAAAAAGCAGGCAGCCTCACCAAGCCGCTACAGCAGCTGGAAACTTAGCCGGTT 2730
TAACAACAGGGCTCAAACCCGGGCCTTGCATCTGCTGGCAAGCACCCCTTGTCTAGTCTACATCCCCAGC 2800
      2810      2820      2830      2840      2850      2860      2870
ACCCTCCATTTGTAAATCTAGGTGGCATTTGTCAAGGTATGTATGTCATGAGCCCGCCGCTGGGCGTTTT 2870
GGATTTGTTCTCTCATGGAAATGGCCCCACCAATGCCTTTGCTGCCCCATTTACAGAGGAGGCGAAAGGC 2940
ACAAAGAAGTGAGACAGCCCGGGGACAAGTCCTCATCCACTCACTCCCCACCATACACGGCCACTCCGCC 3010
ATGCCACCTCCCCTCAGTGTCTAGTGCAGACCCCCTCAAGGGAAATCCCAGACCCTTCCTTTCCAGCCAG 3080
GTTTCTTGGTGACAGAAGGCCCATCCTAATCTTGCTATGCCACAGTGGTGTGAAGGTGCTTGAGCCTGGG 3150
      3160      3170      3180      3190      3200      3210      3220
CAAGCTCAGGCTAGCCCAGAAGAGCAAGGAGGGAGCGATAGATAGATAGATAGATAGATAGATAGATAGA 3220
TAGATAGATAGATAGATAGATGGATGATGGTGTGGCTGAAGGTGTCACTTGGGCATGAAGCACTTGGCCT 3290
CCAGTGTCACATAAATCAGGCATGGTGGTGCAGAACCTCTGGTCCCAGCATCCAGAAGGTGAGGCAAGAG 3360
CAGCAGACATCTAAGGTCAAATGCAGCCATCAGTGAGTTCCAGGCAGCTCATACATAAACAATATAAAAC 3430
CAAGGAAAGGATGTTAAGGTTGAGCAGATTCACCTGGGGCTCTCTGCTGCCATGCTCTGGAGCCCCACCT 3500
      3510      3520      3530      3540      3550      3560      3570
ACAGGACATTTGTCTCCAGCAGTGGCATTTGCTCATGTTTTCTCTGTACTGATGCCTCCCATAACCTGCC 3570
CTTGGAGAATGCTGCTGGGAGCCCCTGGGTGGACATGAGAAAGGTTAGCGAACAGCGCTTGACTGAGAGC 3640
AATTCTGCGGTGCAAATGTTCTGTCTTGTGAATAAGTTATCCATGAGGAGGCACAAGGGCAGACTGTGTC 3710
TGGCCAAGCAAACCCTGGTGTCCCTCCAGGTCCCTGCCCTCCATGCTCAGGGACAAGCCGCGGTTACCAC 3780
TCACCATGCTCTTGTCTCCTTCCCCCAGGAGAGAAGTATGAACTGCATGCAGCGACAGACACCACTCCCA 3850
      3860      3870      3880      3890      3900      3910      3920
GTGTGGTGGTCCACGTGTGTGAGAGTGACCAAGAGAATGAGGAGGAAGAGGAAGAGATGGAGAGAATGAA 3920
GAGACCCAAGCCCAAAATCATCCAGACACGGAGACCGGAGTACACACCCATCCACCTCAGCTGA 3984
```

Figure 25A

10 20 30 40 50 60 70

GAAAAATTCGAGGGACTGTTCCGGACCTATGATGAATGTGTGACGTTCCAGCTGTTTAAGAGTTTCCGAC 70
GGGTTCGAATAAATTTCAGCCATCCCAAATCTGCAGCCCGTGCCCGGATAGAGCTTCATGAGACTCAGTT 140
CAGAGGGAAGAAGCTAAAACTCTACTTCGCCCAGGTGAGTCTTTAACCTGCTGGTTTGGCACAACATTTA 210
GAGGACGTGTTGCTATTGGAGTAGAATCAGATTCAATTTCCAGCATGCACATGGTGGTTCACAAACATCT 280
GGTGCCCTCCTCTGACCCTTTAGGGTACCACACACACACAGACACACACACACACACACACACACACACA 350

360 370 380 390 400 410 420

CATACACACACAGTACATACACATAAGTGTGGGCAATACATTCATGCACATAAATTAAATTTAGAAGTAT 420
AAAAAGTCATTGTTAATTGGAAAATAAATAAATTAAATTAAAATGTAAATGAGGACCTCGGGAGATGGTT 490
ATGCAGTTAAGAAAGCTGGCTGCTCTTCTAGAGGACATGAGTTCGAGTCCTAGCACTCATATGGTGTCTC 560
ATAATTGTTTGTAACCCCTGTTACAGGGGAACCAATGCCTTCTTCTAGCCTCCTACACACCCACAAATAG 630
GTTTGCTGTTACAGTTACTTCACTAAGAAATTAATTTAGTGGTTGTCTAAGACCTGCCCAAGATAAACCA 700

710 720 730 740 750 760 770

GTCAACATTCTAGCATGGAGAGAAAAGGGGACCCTGAGCCCAGACCTCCAACTGAGGGACTTTCAACAG 770
TTGATGGATGCTTGGGGGGGGGATGTTTCCTTGGTGGTTTGGTCTCTGGTAGGTTGAGTATGGTCCAGG 840
GGATGGTCCCACACCCATGCTCATCTGGACAGCACTAACTGGACTCAGCGGATATGAAAACATAAAGAAC 910
ACGAGGAAGGGAAAGGAATGGAAGCAAATCTGATCAAAATATATTTATACATGTATGAAATCCTCCGAGC 980
TATTTATACATGTATGAAATCCTCTGAGCTAATGTTCTTAAAATAAGGAAAGAAACAGACACTGACAGTG 1050

1060 1070 1080 1090 1100 1110 1120

AGTTCCAGATTGAGCAGTATCTGTGTCCTAGGACAGAGGCTCTAAGACCTGCCAAGCTAAGTTCTAACTA 1120
GGACAAGTCTCAGAACCTCACTGGGACTCAGAGTCCTCATCTATAAGATGGCAATGAAGACATTATCAAC 1190
CCATGTAGCTGCTGTGATGGTGACATGGAAAGCTGTGTGCAGCTGTGCCTAGATTTCTGGTAAAGGGACA 1260
ATAATTTCCAGCTAGGAACTGCAACAGAACTGATCTCACCACAGCCGACTCCTAACCTTCCCGACAGGGT 1330
TGTGATTAAAATTTAAATGATATGTTTAATGGTATACTAAATACATTCATGATAAAAAGTTATAAATCCA 1400

1410 1420 1430 1440 1450 1460 1470

TGAAAATTAATTGTATGTTTTGCAAAGCCAAATACTCATTATCCTGAACAGGGATGGGTAGTTCTTAGGG 1470
ATGTTCATGAAGCCCACAGCACTAGTTGTCGGTATTCACTCTCCATCAAGGCCTTATCCATCACTAGGCA 1540
ACAGTCACCTCTCAAGGATGGCTTCAGCTGCTGACTCCTGCTAAAATCCTACATCTCTTATAAATTCATG 1610
TAGCTAGAACAATCTTAGATCATCATTTATTAAAACCTGCATCAGAACTAGTTGTGTCAGCTGTAGACTC 1680
CTGCTAAAATCCTACATCTCTTACAAATTCATGTAGCTAGAACACACTTAGATCATCATTTATTAAAACC 1750

Figure 25B

1760 1770 1780 1790 1800 1810 1820

TGCATCAGGACCAGTTGGCCTGAGGCAGGAGACCTTGCATTCAAGGCCAGCCTGAGCTATCCAGTAAGGT 1820
CCTGTCTCAAAAAGACTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTG 1890
TTTAATATGTGTGTGTGTGTGTGTTTAATATGTGTGTGTATATATATATGATATATGGGTACATAGATAT 1960
ATGATACATACATACATGATAGACACATACATAGATACATACATAGCTATATAGATACGAGAGAGAGACA 2030
GAGAGAGAGATTTCCATTAAAAGATAACATGGAGTTACCATGTGACTCGTAAATTCTCTTCTAGGTTCTA 2100

2110 2120 2130 2140 2150 2160 2170

AAAATCATGAACTCAAACAAATAGTTAAGCAAGAATTCACAGCAGCACTGTTCACAATAGGCCAACAGTG 2170
AGAACTACCTAAAGATCTTCAACAGATAAAGGGATAAAGAGACAATAGTATGTTCACACAAAGGAATATT 2240
ATTCAGCTGAGAGAGAGAGAGAGAGAGATGTTGATAATCCATCACCAAATAATGGGCCTTTAAAATGCAA 2310
TGGAAGCTAGACACAAAAGCTCATCTGTTCTGTGGTTCCATTCTCATAAAAGAGTTAGATAAGTTCAGAG 2380
AAGTAGACACAGCTTGACAACCATCAGGGGTAGTAGGAAACTACATTAGTAGTCGTTATTTAAGGGATGC 2450

2460 2470 2480 2490 2500 2510 2520

ATAGTTGGGGTTGGAGAGATGACTTAGCAGTTAAGAGCACTGAATGCTCTTTCGAAGGCCCTGAGTTCAA 2520
ATCCCAGCAACCATATGGTGGCTCACAACCATCCATAATGAGATCTGATGCCCTTTTCTGGAGTGTCTGG 2590
AGACAGCTACCATGTACTCACATATAATAAATAAATAAATCTTTTTAAAAAAGGGGTGTGTGTGTGCATA 2660
GTTATCCTTAGAGCCATGGAAGGGTTAGGGTAGTTGTTCTCAACCTTCCTAATGCTGTGACCCTTTAAT 2730
ACTCATGAAGTGGTGATCCCCAGCCACAAAATCCTTTTCGTTGCTGCTTTATAACTGTAATTTTGCTAGT 2800

2810 2820 2830 2840 2850 2860 2870

ACTATGAATTATGATACCACTGTGTGTGTTTTCTGATGGTCTTAGGCAGCACTCTGGCTTGCTCACCATC 2870
TAGCCTAACCTTATTGGTGAGATCAAGGTTTTTGGGTTGTTTTTTTTTAAAGGGCACTGTTACCTAAGGA 2940
AGGACATTAGAAGTTGTCCACTAGCTTCCACATGTACACACTCATAAGGGCACACAATGTAGTACAGGGC 3010
TTGGTGACCCTGGTGCTCATTAAAAGATGGAAACTGCTGTTTTTTTAGGATTATGTAAACAATGAGTTCA 3080
GCACACTGTGTACTGTAAGGAGTGATTGCTACTGCAGTCTGGCCCTCAGTGAAGCCCTGCCCAGCTGCAA 3150

3160 3170 3180 3190 3200 3210 3220

GCATGGACAATCACATGTCTCATTATCTTGTTTGAAAGGTCCAGACCCCAGAGACAGATGGAGACAAACT 3220
GCATTTGGCACCTCCACAGCCTGCCAAACAGTTCCTCATCTCACCCCCTTCATCTCCATCTGTTGGCTGG 3290
AAGCCTATCAGCGATGCCACACCAGTCCTCAACTATGACCTTCTTTATGCTGTGGCCAAACTAGGACCAG 3360

Figure 26A

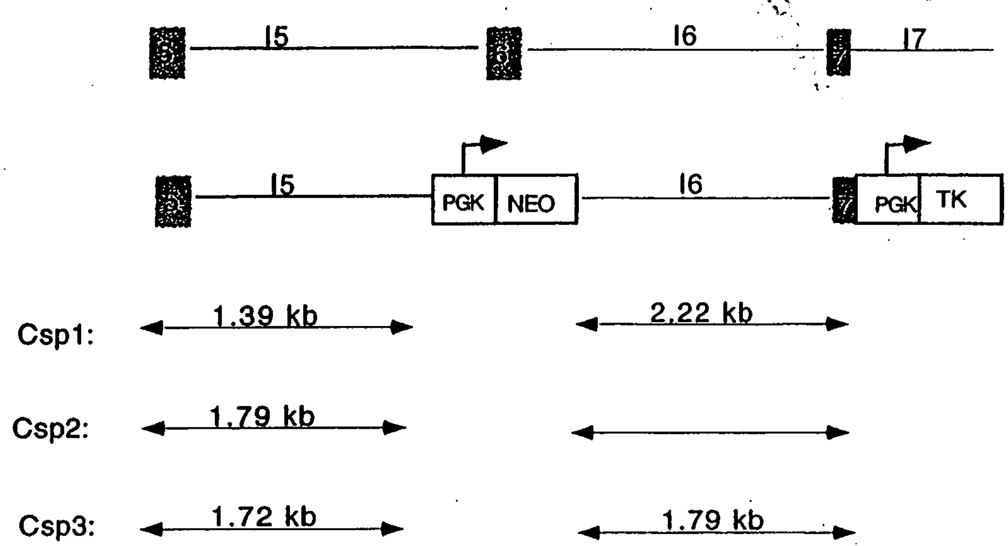
```
        10        20        30        40        50        60        70
CGGAAGCTGAAGCTTTACTTCGCACAGGTAATGGCCGTTCTGCGCCTGCGCACACAGCCTGCTCCAGTTC 70
CCGCTCCAGCACGGGGTCAGAGGTCTGTGAGGTCAGCAGTCACGTGAGCCAGGGCTGCCGTGCTTTTTCT 140
GACTTTACACATACGTCATTTCATGTATTTTAGGAGCACATTAAGCCTCTGTTCATGTTTCTCTGAGACG 210
AACACCTAAGGGGTTCATTTTTCTGGCGATTTTGCTCAGCTAGGGCTCTGTGAGGGAAGTCCTGATACTT 280
CGAAGTTGGCAGATTAAACACTGTGCATCTAAAATGGCACCGAGGACATGACATCCGTGGGAAAACAGAA 350
       360       370       380       390       400       410       420
CAAAACCTTCAAGGGTCATCAAGATGGCCCAGGGGGTGAAGGTGCTTGCCACCAAGCCTGGCAGCCCGAG 420
TTTGATCCCAGGAACTCATCCACGGGTGGAAGGAAAGAACCAACCTGTGTCCTCTGAGGACCACATATGC 490
AGTTTTCTCTCTTCTGAGACAGTAGTGTGTTAGTCAGCCCTTCCCAGCGAATTAGTTACTGGGATGAGAC 560
ACTGTGACCAAAAGCACCCAGGAGACAAAAGGTGTATGTACTTTACTTATAATGAATCACCATTCATTGA 630
GGGAAGCCAAGGCAAGAACTCAACCTGGGCAGAAACCTGGAGGCAGAGGCCATGGAGGGGCGCTGTTTAC 700
       710       720       730       740       750       760       770
TGGCTCCTCATGGCCTACTCAGCCTGCTTTCTTTTTTTTGTTTTGTTTTTTGTTTTTTGAGACAGGGTTT 770
CTCTGTATAGCCCTGGCTGTCCTGAAACTCACTCTGTAGACCAGGCTGGCCTCGAACTCAGAAATCCGCC 840
TGCCTCTGCCTCCCGAGTGCTGGGATTAAAGGCGTGTGCCACTGTGCCTGGCTTCAGCCTGCTTTCTTAT 910
AGAACCTAGAACCACAACCCAGGCTGGTATCATCCACAGTGGGCAGGGCCTTCCCCACATTGGTCACTAA 980
GAAAACTTCCTGCCTGCAGTCAGGTCTTCTGGAGACATTTTCTCAGTTGGGTTCCTGTCTCTTGATGACT 1050
      1060      1070      1080      1090      1100      1110      1120
AAAGCTTGCATCAGGTTGACATATAGTAGCCAGCACACCCACTCACACCACTAGCAAATACCTGGGAGAG 1120
TCAGCTGTAAAGGAGAAAAGTCTCGGCTTGTGGTTTGCAGGTTTCAGTCTGCATGTGATTGGCACTTTTC 1190
CTGTGAGCCTGCTGTGCAGTAGCACATAGGGGCAGAGCAAAGCTCTTCACTTCGTTCATGGGAAGCAGGA 1260
AGAGTAAGGGGTTGGGGTTCCACTGTCCCTTAGGGTATGTCCCCATGACTAAAGGCCTCCCTGCCTCCTG 1330
AAGGCTCCCAGTTTGACCTCTCAGGGGAGCAAGCCTCTATTTACTATGTAGAGCCCAAGGGTCACTTAGA 1400
      1410      1420      1430      1440      1450      1460      1470
GCCCAGACCACAGAGTAGCACGTTTATCAAGGGTCCAGGGCCTGTGGCCACTTCCAGTCCACCACCTGGA 1470
AGGTCACAGACAGTTTGAGAGACAGTTTTAATCACCCCTCCAAGAAAGTAACAATTACCATAAAGTTGGA 1540
AATGAAAGCCCTGTGGTGATGGTGCAGGCCTTTAATCTAAGAACTGGAGGCAGAGACCGTGAGATCTGTG 1610
AGTCAGGCCTACAGAGTGAGTTCCAGGACAGCCAGGGATACACGGAGAAACCCTGTCTCAGAAAAAGAAA 1680
AGAAAGGACAGCTGCTCACAAGCACGCCTTTCCCTGCAGGTGCAGGTGTCCGGGGAGGCCCGggacaagt 1750
      1760      1770      1780      1790      1800      1810      1820
cctacttaCTGCCACCACAGCCCACCAAGCAGTTCCTCATCTCCCCTCCCGCCTCACCCCCCGTGGGGTG 1820
GAAGCAGAGTGAAGATGCAATGCCAGTGATCAACTATGACCTGCTCTGCGCTGTCTCCAAGCTGGGCCCA 1890
GGTACTGCATTCCACCTTCGCTCTCCGCGTCCTCGGACATTGCTGTTCTGTGTGTTGGAGACTGTGTGCA 1960
GTATGGGGTGCAGAGCCCAGCAACACCAGCACCGTCCAGTGGGCGGTGTGGCCACACCAGTCTGAGTTCA 2030
CACTCGAGCTGTACACTTTCCAGTGCTGTGGTCCTCAGCCAGTTGCCTAGCCTGGGTTATCTGAGTGTGT 2100
```

Figure 26B

```
        2110      2120      2130      2140      2150      2160      2170
TCTAAGGATTAAACGCTGTCTGCAGCGTGATAACTTTAGCCATTCAGCCAGAAGTTAATATAGGCGGTTA 2170
GTGAACATCCTCACTGCTTTCTCTCTGCAAGCCAGTCAGCACAGTGTCTGTCGTTTGGCAGCTGCTTTGG 2240
GTGACAGTGACAATGACCTATCGCCCTTCCAAAGTTCTATCTCTCTCTCTTTTCACTTCTTACTTCCTTC 2310
TTTTCTTGCTCGGTCTCACTCATCTTTAATACTGCAAGAAGCCGATTCTTCTAGGGCACTTCAGAGGCTT 2380
TTGAGAAGGCACTCTATGCTCCTGGGCGGNTGAGCTCTTCGATGGCAGAGGCCCTACCGTAGACACCGCT 2450
        2460      2470      2480      2490      2500      2510      2520
GCCTAGAGCTTAGCCAGTGCCTCCCATGGCGCCCCAACACCACTGTGAATTTAACTATCCCACCTTAGTT 2520
ATCTATAGAACAGCAGTTAGCATTTATATTAACATTTTAATTAGTATTTATGTAATATAATCAATGGGTT 2590
CTCGTCTTCTTCCTGAGCACAAAGCCAGAGTAAGCATAGAACAGAAGAGACAAGAAGAGAAGAGATAGGA 2660
AGAGACAGGAGCTGTTTGCAAAGCAAGCCCTCCCCGAGTGAAGGAAGCTGTGTATATTCATACAGTGGCA 2730
TGTGCACTCCTGAGCACGCGCAGTTGAAAATCATGGAGATGAACATGGTGGACAGGGTGTGCTTGGGTTC 2800
        2810      2820      2830      2840      2850      2860      2870
GCTTGCACCATGAAGTTTCACTTGAAAATAAGAGAAGGATGGTTTTAAGGTGTGTGCTAACAGGAGTCTG 2870
CCTTGAAGGTGCCTGAAGTGCTTGGATTTAACTCCTAGGGCTCAGGACAGAAGGGACGGTGTCTTTATTT 2940
ATTTTTTTTTAAGACTTATGTATATGAGTACATTGTAGCTGTACAGATGGCTGTGAGCCTTCATGTGGTT 3010
GGGAATTGAATTTTTAGGACCTTTGCTTGCTCCCATCAACCCCTCTCGCTCTGGTCGGCCCTGCTCGCTA 3080
GTCCCTGCTTGCTCCAGCCCAAAGATTTATTTATTATTATATATAAGTACACTGTAGCTGACTTCAGACG 3150
        3160      3170      3180      3190      3200      3210      3220
TACCAGAAGAGGACATCAGATCTCATTGCGGGTAGTTGTGAGCCACTATGTGGTTGCTGGGATTTGAACT 3220
CTTCGGAAGAGCATCAAGTGTTCTTACTCACTGAGCCATCGCATTAGCCCGACAGTGTCTTTACAAATAG 3290
AATTTCTGCAGGGCATGGTGGTACTCAACTTTAACAGCACTTGGGAGGCAGAGGCTGGCAGCTCCCTGGG 3360
AGTTCCAGGTCAGCCTGTCTACACAGTGAGCCTAGGCCAGCCTGGGCTACATAGTGCGACTCCAGGGAGT 3430
TTTTGTTTTTGTTTTTGTTTTTTTTAAATGCCAGCACTTGGGAGATGGAAGCAGAAGAATTAGAGTTCAA 3500
        3510      3520      3530      3540      3550      3560      3570
GGTCAGCCTCAGCTACAGCAGCAAGTTTCTAACTGGCCCAGATTTCATGAGACGCAGTCTTAAAAAAAAA 3570
AAAAAAAAATCAGCCACTGAATGACGTAGTAGAAGAGGAAGTTGGGAGATAGAAGAACTTGATTTCCTTC 3640
ACTGGGAGTAAGGCTCCTTCCTGTGCTTGCAGGGGAGAAATACGAACTGCACGCGGGAACCGAGTCCACC 3710
CCCAGTA 3717
```

Figure 27

Schematic Representation of the Gene-targeting Vectors Used to Disrupt the Csp1, -2, and -3 Genes

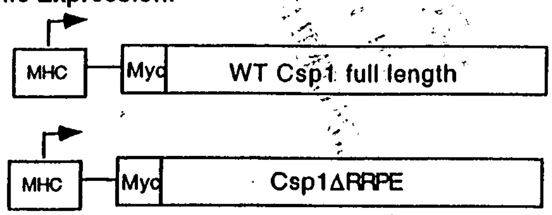


This schematic diagram shows the organization of the Csp genes (top) and the targeting vectors (middle)constructed to disrupt the Csp genes. Our targeting vector will replace exon 6 with the neomycin drug resistance genes. This exon contains the start of the inhibitory, or c-terminal domain of all three genes which should effectively destroy the calcineurin inhibition activity. The genomic structure of all three genes is relatively similar with different size introns (I5, I6). Exons are denoted by the shaded boxes with numbers.

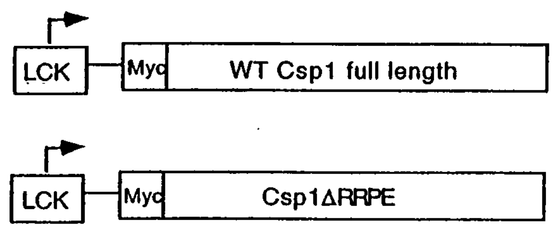
Constructs Used to Generate Tissue-Specific Expression of Csp1 in Transgenic Mice

Figure 28

Cardiac Specifc Expression:



T-Cell Specific Expression:



This schematic diagram demonstrates the constructs injected into blastocysts to generate transgenic mice. Wild-type full length myc-tagged Csp1 under the control of a myosin heavy chain (MHC) promoter (top half) will ensure cardiac specific expression. Similarly Csp1 with the sequence element, amino acids,188-191,"RRPE" deleted is also expressed under the MHC promoter.

Myc-tagged wild type Csp1 and Csp1ΔRRPE are also expressed under the LCK promoter which will ensure T-cell specific expression (bottom half).